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OBITUARY.

We greatly regret to record the death, on July 23, of Sir William Ramsay, K.C.B., F.R.S., an honorary member of the Society. An obituary notice will appear in a forthcoming issue of the Journal.

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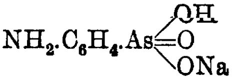
EXCRETION AND SECRETION OF SALVARSAN AND NEO-SALVARSAN.

By JOHN WEBSTER, F.I.C.

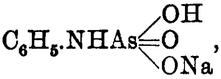
(*Read at the Meeting, May* 3, 1916.)

Salvarsan ("606") (or Kharsivan, as the English preparation is termed) and Neo-Salvarsan (or Neo-Kharsivan) are at the present time probably the two most effectual remedies for the treatment of syphilis. With regard to the evolution of these valuable drugs, it may be stated that the goal which the experimenters had in view was a drug that would be non-toxic to the patient, though powerfully toxic as to the parasite (*Spirochæta pallida*)—that is, parasitotropic, but non-organotropic.

After much experimentation it was found that compounds of arsenic with the benzene nucleus gave good results.



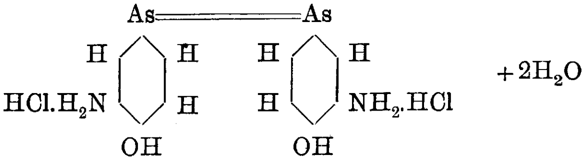
Atoxyl (sodium paramino-phenyl arsenic acid) $NH_2.C_6H_4.As{<}\substack{OH \\ =O \\ ONa}$ was such a compound. This drug gave apparently most promising results, as its action on the parasite was strong, and it had a low organotropic action. But it was eventually found that atoxyl had not (at any rate in all cases) so low an organotropic action as had been hoped. It had occasionally a marked action on the optic nerve, causing in some cases total blindness.



With regard to atoxyl, it is important to note that it was originally supposed to have the following formula: $C_6H_5.NHAs{<}\substack{OH \\ =O \\ ONa}$, that is to say, the amino group was directly bound to the arsenic. It was after it had been proved that the amino group was not directly bound to the arsenic that various substitution products, such as salvarsan, were evolved.

Salvarsan ("606") was found by Ehrlich and his assistant Bertheim to be the most satisfactory compound at the time. This compound (diamino-dihydroxy-arseno-benzene-dihydrochloride), obtained from atoxyl, was proved to have very high parasitotropic action, but very low organotropic action, and to have no such effect on the optic nerve as was the case with atoxyl.

The formula of salvarsan was originally stated to be $C_{12}H_{12}N_2O_2As_2 2HCl$, requiring 34 per cent. of arsenic. Gaebel, however (*Arch. Pharm.*, 1911, **249**, 241-247; *Apoth. Zeit.*, 1911, 215), has shown that the correct formula of salvarsan is $C_{12}H_{12}N_2O_2As_2.2HCl + 2H_2O$, requiring 31·6 per cent. of arsenic. Graphically it may be expressed thus:

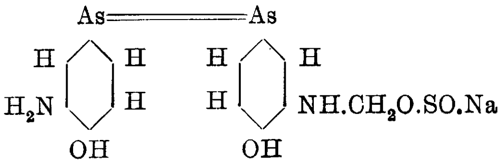


Chemical Tests.—Salvarsan readily gives the Reinsch test. The statement that salvarsan responds to the Marsh test (Gaebel, *J. Chem. Soc.*, 1911, Abstr. II., 448) is misleading. It is true that a slight mirror is obtained from relatively very large amounts of salvarsan, but the test is unsatisfactory if performed direct—*i.e.*, without previously decomposing with nitric and sulphuric acids.

Salvarsan may be distinguished from inorganic arsenic compounds by the following tests (Gaebel): (1) Hydrogen sulphide gives no precipitate with salvarsan, even after previous boiling with hydrochloric acid. (2) Bettendorf's reagent (solution of stannous chloride in hydrochloric acid) gives a yellow precipitate, soluble on warming, and reappearing on cooling. (With inorganic arsenic the reagent gives a brown coloration or brown precipitate.)

The organic part of the salvarsan molecule may be detected by colour reactions given by the diazo-derivative with (1) α-naphthylamine, or (2) resorcinol (Abelin, *Münch. Med. Wochenschr.*, 1910, **58**, 1002; see ANALYST, 1911, **36**, 452).

Neo-Salvarsan (or *Neo-Kharsivan*).—This compound was the result of an endeavour to obtain a drug similar to salvarsan in its physiological and therapeutic effects, and yielding a neutral solution. *Salvarsan* gives a solution acid in reaction, and before injection it is neutralised with sodium hydroxide, which throws down a precipitate; more sodium hydroxide is then added until the precipitate is just dissolved. The disadvantage of the alkaline solution prepared in this manner was eventually removed by the introduction of a methylene sulphoxy radicle into the molecule, resulting in the production of neo-salvarsan ("914"), having the formula



Neo-salvarsan is a yellow powder readily soluble in water, forming a neutral solution. Its physiological and therapeutic effects are similar to those of salvarsan. Owing to the introduction of the further group into the molecule, its arsenic content is two-thirds that of salvarsan; the corresponding dose of neo-salvarsan (having regard to the arsenic) is therefore one and a half times that of salvarsan.

Excretion and Secretion.—Salvarsan and neo-salvarsan are excreted fairly rapidly at first, the amount excreted *per diem* falling, as a rule, rapidly after about the second day, until at the end of about a week the quantity excreted is comparatively small (though the salvarsan may possibly still be detected by the α-naphthylamine or resorcinol test); arsenic may still be found in appreciable amount in the urine at the end of fourteen days, and, as a rule, can be detected at the end of three weeks, or even longer.

The following analyses show that in some cases the daily quantity excreted is not a rapidly diminishing one (Cases III. and IV., in which at the end of a week relatively large amounts of arsenic were still being passed).

In each of the following tables of urine analyses, column 1 represents the mgrms. of arsenic (calculated as As_2O_3) present in 100 c.c. of urine, and column 2 the mgrms. of arsenic present in the total volume passed in twenty-four hours. The analyses were carried out in every case on the twenty-four hour specimen.

Case 1.—0·6 *grm. Salvarsan given.*

Number of Days after Injection.	(1) Mgrm. As_2O_3 in 100 C.C. Urine.	(2) Mgrms. As_2O_3 in Total Twenty-four Hour Quantity.
1	1·0	2·8
3	1·0	3·7
5	0·17	0·73
7	0·17	0·22
8	0·07	0·35

Case 2.—0·5 *grm. Salvarsan given.*

Number of Days after Injection.	(1)	(2)
2	0·66	9·0
4	0·30	0·69
7	0·30	0·51
10	0·15	0·93
13	0·03	0·21

Case 3.—0·5 *grm. Salvarsan given.*

Number of Days after Injection.	(1)	(2)
2	0·2	3·1
5	0·2	1·7
8	0·15	2·1
11	0·05	0·38

Case 4.—0·5 *grm. Salvarsan given.*

Number of Days after Injection.	(1)	(2)
2	0·4	2·1
4	0·2	1·4
7	0·2	1·9
10	0·15	0·47
13	0·10	0·91

Case 4 (*continued*).—*Another* 0·5 *grm. Salvarsan given.*

Number of Days after Injection.	(1)	(2)
4	0·4	2·6
7	0·4	2·5
10	0·2	0·40
13	0·1	0·44
16	0·03	0·16
21	0·025	0·16

Case 5.—0·5 *grm. Salvarsan given.*

Number of Days after Injection.	(1)	(2)
3	1·3	8·8
5	0·8	5·9
8	0·33	3·1
11	0·12	1·3

Case 6.—0·5 *grm. Salvarsan given.*

Number of Days after Injection.	(1)	(2)
2	1·3	8·3
4	0·4	1·3
7	0·16	0·7
10	0·11	1·1
13	0·025	0·15

ANALYSES OF ABDOMINAL VISCERA, MUSCLE, AND BLOOD.

In cases of death following the injection of salvarsan and neo-salvarsan, arsenic is found in relatively large amounts in the liver, kidneys, and spleen, and in small amount in the muscles and blood. It is to be noted that arsenic is entirely, or almost entirely, absent from the brain and nervous system (any effect, therefore, on the nervous system is not due to the presence of salvarsan as such).

Below are given the results of analyses of various organs in nine cases of death following salvarsan treatment.

Case 1.

Aged twelve. Two doses 0·3 grm. salvarsan (one week interval). Death eight days after second injection.

	Mgrms. Arsenic per 100 Grms.
Liver	2·5

Case 2.

Aged twenty-three. Two doses 0·4 grm. salvarsan (four days interval). Death three days after second injection.

	Mgrm. Arsenic per 100 Grms.
Liver	0·83

Case 3.

0·5 grm. salvarsan given. Death twenty-nine hours later.

	Mgrms. Arsenic per 100 Grms.
Liver	4·0
Brain	0·01

Case 4.

Aged thirty-six. Two doses 0·6 grm. salvarsan (two weeks interval). Death two days after second injection.

	Mgrms. Arsenic per 100 Grms.
Liver	1·1
Kidney	0·2
Spleen	2·0
Brain	None detected.
Spinal cord	,, ,,
Aorta	0·025
Stomach contents	0·08

Case 5.

Aged nineteen. 0·6 grm. salvarsan given. Death nine days later.

	Mgrms. Arsenic per 100 Grms.
Liver	0·16
Kidney	0·13
Spleen	0·17
Spinal cord	? Trace
Small intestine	0·1
Large intestine	0·11

Case 6.

0·9 grm. neo-salvarsan given. Death four and a half hours later.

	Mgrms. Arsenic per 100 Grms.
Liver	0·8
Kidney	1·0
Brain	0·02
Blood	0·05
Muscle	0·06
Bone	0·07

Case 7.

Dog weighing 24 lb. 3 oz. 0·3 grm. neo-salvarsan given. Animal killed twenty-four hours later.

	Mgrm. Arsenic per 100 Grms.
Liver	1·0
Kidney	0·4
Brain	Trace only.
Blood	0·1
Muscle	0·08
Bone	Trace only.

Case 8.

Two doses 0·9 grm. neo-salvarsan given (one week interval). Death four days after second injection.

	Mgrms. Arsenic per 100 Grms.
Liver	0·2
Kidney	1·5
Suprarenal gland	? Trace
Brain	Trace only.
Blood	0·02
Muscle	0·03
Bone	Trace only.
Aorta	None detected.
Skin	,, ,,

This case is of special interest. It will be noted that the amount of arsenic found in the kidney was relatively very high. The patient when admitted to hospital was suffering from acute nephritis.

Case 9.

Aged three weeks. Three doses of 0·02, 0·04, and 0·04 grm. respectively of salvarsan were given, with intervals of two days and one day. Death on day following last injection.

	Mgrms. Arsenic per 100 Grms.
Liver	0·4
Kidney	0·49
Spleen	0·39
Suprarenal gland	0·17
Lung	0·03
Brain	None detected.

PRESENCE OF SALVARSAN IN THE BLOOD AFTER INJECTION.

It has been stated (McIntosh and Fildes, *Proc. Roy. Soc.*, B, 1914, **88**, 324) that the blood is practically free from arsenic two days after an injection of salvarsan.

An experiment which I carried out points to this not being so in every case.

I have examined blood removed from a patient twenty-four hours, forty-eight hours, and five days, after intravenous injection of 0·9 grm. of neo-salvarsan, and found respectively $\frac{1}{5}$ mgrm., $\frac{1}{10}$ mgrm., and $\frac{1}{40}$ mgrm. of arsenic per 100 c.c. of blood.

With regard to the presence of arsenic in the blood after salvarsan injections, it is to be noted that the arsenic is confined entirely, or almost entirely, to the serum.

In one case examined after injection of 0·6 grm. of salvarsan the blood was drawn off at the end of one hour, and the clot separated from the serum.

The serum was found to contain 0·5 mgrm. of arsenic per 100 c.c. serum.

In the case of the clot, 28 grms. gave only a faint trace (less than $\frac{1}{300}$ mgrm.), this trace being possibly due to incomplete separation of the clot from the serum.

THERAPEUTIC INFLUENCE OF THE MILK OF LACTATING WOMEN AFTER TREATMENT BY SALVARSAN OR NEO-SALVARSAN.

It had been observed by Taege, and later by Duhot, that beneficial results had apparently been obtained from the ingestion by a syphilitic child of milk from the mother who had undergone treatment by salvarsan. This could be explained either (1) by the effect of the presence of salvarsan in the milk, or (2) by antibodies present in the serum of the mother.

Experiments carried out on human milk in two cases of salvarsan treatment point strongly to the latter view.

Case 1.

The mother received three injections of 0·4, 0·5, and 0·6 grm. of salvarsan at intervals of three days and five further days. The child was suckled the whole time. All symptoms of syphilis in both mother and child were stated to have cleared up within five days of the second injection.

Specimens of milk were taken (1) seventy-two hours after the first injection and (2) twelve hours after the second injection.

The former showed a faint trace of arsenic (less than $\frac{1}{100}$ mgrm. per 100 c.c. milk); in the latter no trace of arsenic was detected.

Case 2.

The mother received an injection of 0·5 grm. of salvarsan.

Samples of milk taken five and a half, ten and a half, sixteen, thirty-six, and sixty hours respectively after the injection showed no trace of arsenic by the Marsh test.

In all the above analyses the method employed for the estimation of the arsenic was treatment with nitric and sulphuric acids and subsequent reduction by potassium meta-bisulphite. The resultant solution was then used in the "electrolytic Marsh" apparatus, and the mirror obtained compared with standard mirrors prepared from known quantities of arsenic. The whole of the arsenic found was considered to be derived from salvarsan or neo-salvarsan respectively.

In conclusion, I desire to express my thanks to Colonel W. H. Willcox, A.M.S., in whose laboratory at St. Mary's Hospital I have been enabled to conduct these analyses, and to whom I am indebted for the cases detailed above.

DISCUSSION.

Mr. A. CHASTON CHAPMAN said that one would not expect substances having the constitution of salvarsan or neo-salvarsan to be directly reduced in the Marsh apparatus, though in the Reinsch test one would expect to get the arsenic deposited on the copper. He would be interested to hear the exact procedure adopted in determining such very small quantities of arsenic occurring in such considerable quantities of viscera as must necessarily have been dealt with. For instance, in Case 3 0·01 mgrm. of arsenic was found in the brain. Although brain tissue was perhaps not peculiarly difficult to oxidise, yet in treating such a large quantity with nitric acid and sulphuric acid it would be reasonable to expect a considerable error. In the case of the liver the oxidation difficulties presumably were still greater. According to his own experience of the oxidation of organic matters in general with nitric acid and sulphuric acid, very low results were apt to be obtained, and those who were concerned to any considerable extent with the estimation of small traces of arsenic considered it, as a rule, desirable to avoid the destruction of organic matter whenever possible, as in many cases it was, though of course in such cases as those now under consideration it obviously was not. With a lead or cadmium cathode the sensitiveness was greater, but still not so great as with zinc and hydrochloric acid, and still less than when the zinc had been coated with cadmium. He could quite understand that for some purposes extreme sensitiveness was, as Mr. Webster had said, not necessary, but when the quantities of arsenic were so small as in some of these cases, he should venture to think that it would be best to employ as delicate a method of estimation as might be possible. From a chemical point of view it would have been interesting if some more information could have been given as to the methods to be adopted for the detection of salvarsan and its discrimination from, say, arsenious oxide or other inorganic forms of arsenic. Tests based on diazotisation had been suggested, but it seemed uncertain whether the diazo compounds of salvarsan or neo-salvarsan gave rise to such characteristic colours as would enable them to be distinguished from certain other bodies that might be obtained on extraction with alcohol.

Mr. R. BODMER said that he had had a good deal of experience of the estimation of small quantities of arsenic in toxicological cases, but he must say that the accurate estimation of such small quantities as 0·01 mgrm. in 100 grms. seemed to him a rather difficult matter. The extreme delicacy of the zinc and hydrochloric acid method was, of course, well known, but he had always found it difficult owing, on the one hand, to the possibility of the zinc being insensitive, and, on the other, to the difficulty of getting zinc and hydrochloric acid absolutely free from arsenic. Quite recently he had been obliged to reject three different samples of zinc supplied as arsenic-free by one of the largest chemical dealers, and they were now unable to supply any which could be depended on. The electrolytic method, even though it might be a little less sensitive, was more reliable for work in connection with legal cases. He should like to ask Mr. Chapman how he avoided the destruction of organic matter, and whether, in his opinion, minute traces of arsenic could be satisfactorily estimated if the organic matter were undestroyed.

Mr. CHAPMAN said that if the arsenic were present in the form of arsenious oxide, it did not matter very much what quantity of organic matter was present; in fact, a little organic matter would increase the sensitiveness of the test. On the other hand, if the arsenic were in a form like that of salvarsan, it was obviously necessary to break up the organic compound. In a certain class of compounds with which he happened to have had to deal, and which contained nitrogen as well as traces of arsenic, he had found that on distillation with hydrochloric acid and a little ferrous chloride the arsenic came over still combined with nitrogen, and it was very difficult to get it in an uncombined state. In such cases the results would be low, unless the zinc was exceedingly sensitive. To ensure this he invariably adopted the method, which he had worked out some years ago, of coating the zinc with cadmium. If this were done, the question of "insensitiveness" of zinc practically disappeared. As to hydrochloric acid, he had never found the slightest difficulty in getting acid that would run for the greater part of an hour without showing the faintest trace of arsenic.

Mr. W. PARTRIDGE asked whether Mr. Webster could say in what form the salvarsan would be excreted. In some of the cases in which death followed the administration of salvarsan there seemed to be some doubt as to whether it was actually due to liberation of arsenic in the tissues.

Mr. WEBSTER said that diazotisation tests were not absolutely distinctive for salvarsan. Atoxyl gave almost the same colour with α-naphthylamine as salvarsan. With β-naphthylamine, however, atoxyl gave a brilliant colour, while salvarsan gave practically none. With resorcinol a brilliant red colour was given by salvarsan after diazotisation. He had found urine a week after the injection of salvarsan to give an appreciable colour with resorcinol or α-naphthylamine, although there was no coloration given by the urine before the injection. Such tests, of course, were not very delicate; arsenic would be found in the viscera or urine a long time after the diazotisation test ceased to give a coloration. With regard to the electrolytic method, this was admittedly less sensitive than the zinc and hydrochloric acid method. With platinum electrodes, which he was in the habit of using, the limit of sensitiveness in the ordinary way appeared to be about $\frac{1}{500}$ mgrm.; with lead electrodes it was stated to be about $\frac{1}{1000}$ mgrm.

Mr. CHAPMAN remarked that $\frac{1}{500}$ mgrm. was about the safe limit with zinc and hydrochloric acid, and it would be risky to reckon on such a degree of sensitiveness in the case of the electrolytic method.

Mr. WEBSTER said that, in his experience at any rate, $\frac{1}{300}$ or $\frac{1}{400}$ mgrm. might be reckoned upon in the electrolytic method. As to the quantities of material used and method employed, from 30 to 50 grms. of material were treated with nitric acid and sulphuric acid. That some loss occurred must be admitted, but the conditions were kept as nearly as possible the same in all cases, and the results, though admittedly rather low, were valuable for purposes of comparison. With regard to the zinc and hydrochloric acid method, he could never rely upon the zinc being absolutely free from arsenic, and should not care to use the method for medico-legal cases. With regard to Mr. Partridge's question, he did not think it was definitely known in what way salvarsan acted. It might be mentioned, however, that both salvarsan and neo-salvarsan must not be used unless perfectly fresh; there must be no delay in using the contents of a tube after it had once been opened; otherwise there was great risk of toxic action. With regard to the form in which it was excreted, as already stated in the paper, urine passed several days after injection of "salvarsan" gave the diazo- and resorcinol tests for the benzene nucleus, and presumably some, at any rate, of the salvarsan was excreted as such, arsenic still being found in appreciable amount also.

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